

POLLEN TETRAD SEGREGATION IN *ASTROLOMA PINIFOLIUM* AND IN
ACROTRICHE FASCICULIFLORA.

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(Four Text-figures.)

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Synopsis.

An analysis is made of tetrad segregation in the two species on the bases of three hypotheses— independence, the operation of cytoplasmic competition, and a relationship to the two divisions of meiosis. It is concluded that both cytoplasmic and meiotic conditions may contribute to the behaviour. There is also a significant contribution by environmental factors, and a precise partition of the effects of the environment, the cytoplasm, and the nucleus, is not yet possible.

INTRODUCTION.

Pollen tetrad segregation, in which some or all of the pollen grains in a tetrad are aborted, has been recorded in twelve species of the Styphelieae (Smith-White, 1959). From a consideration of the patterns of pollen development found in other species of the tribe, it has been inferred that this tetrad segregation must have originated on several occasions and that these origins have been dependent upon a prior evolutionary history of monad-type pollen development and cytoplasmic polarity in pollen mother cells. In fact, it is inferred that within the Styphelieae there has been preadaptation to the establishment of pollen tetrad segregation.

It is reasonable to expect that the characteristics of such behaviour might reflect its causation. An analysis of the frequencies of the five possible kinds of pollen tetrad—nullads, monads, dyads, triads and full tetrads—may contribute to an understanding of the mechanisms involved. Unfortunately, the tetrahedral arrangement of the pollen grains in the tetrads, and the distortion due to the differential growth of good and aborting grains, prevent the recognition of the planes of the first and second meiotic divisions. Analysis must be made on the basis of unordered tetrads.

In this paper an analysis has been attempted of data for two species, *Astroloma pinifolium* Benth. and *Acrotriche fasciculiflora* Benth.

Ast. pinifolium occurs in coastal districts of eastern Australia, extending from southern Queensland to eastern Tasmania. In this distribution it shows several major disjunctions, and it usually occurs in small isolated local populations in each region. Still more isolated populations are found in the Warialda district of north-west New South Wales and in the Grampians of south-western Victoria. The pattern of distribution suggests that the species is old and relict. Material studied in this paper was obtained from the east coastal region, from Sydney to Evans Head. Data from Grampians plants are presented for comparison and contrast.

Acr. fasciculiflora is found only in south-eastern South Australia, its range extending from the Adelaide Hills and Mt. Lofty Ranges to Kangaroo Island. The material studied was collected in Long Gully, Belair National Park.

THE DATA.

Observed frequencies of tetrad types for a representative selection of plants of the two species are given in Tables 1 and 2. Table 3 summarizes the analysis according to two hypotheses for 57 plants of *Ast. pinifolium*. Tables 4 and 5 present data and analysis for within-plant variation for two selected plants of *Ast. pinifolium*.

The data given for each plant in Tables 1 and 2 are composite in that they were obtained from two or more flowers and 10 or more anthers. There is significant variation between flowers and between anthers within plants, which is illustrated by the data given in Tables 4 and 5, and by the graph in Text-figure 3.

TABLE 1.
Astroloma pinifolium.
 Tetrad Segregation Data.

Plant and Hypothesis.	Tetrad Type Proportions. % × 100.					N.	(p+q) or (x+y+z).	χ ² .
	r ₀ .	r ₁ .	r ₂ .	r ₃ .	r ₄ .			
R57/8 ..	7673	1790	484	52	1	9595	0.927+0.073	378.2
I ..	7384	2326	273	15	0			
III ..	7658	1808	485	44	5			
LP57/4 ..	7183	2347	415	54	0	4651	0.8751+0.1033+0.0216	0.49
I ..	7056	2571	351	21	0			
III ..	7174	2338	444	41	2			
W54/6 ..	6011	3151	750	83	5	5878	0.847+0.138+0.015	2.2
I ..	5916	3319	698	65	2			
III ..	6006	3147	753	89	5			
R57/5 ..	4982	3538	1262	208	9	4468	0.877+0.123	12.3
I ..	4746	3888	1195	163	8			
III ..	4970	3596	1214	204	16			
TG57/4 ..	4065	4182	1488	243	22	8785	0.705+0.255+0.040	2.7
I ..	4116	4091	1524	253	16			
III ..	4116	4091	1524	253	16			
LP53/3 ..	3998	3728	1866	374	34	8022	0.801+0.199	5.2
I ..	3797	4160	1709	312	21			
III ..	3969	3780	1782	420	49			
TG57/2 ..	3157	4204	2116	482	41	9595	0.642+0.318+0.040	5.2
I ..	3145	4219	2122	472	40			
III ..	3165	4204	2114	477	41			
O55/2 ..	2914	4313	2301	451	21	6088	0.785+0.215	63.1
I ..	3015	4275	2210	515	45			
III ..	2884	4435	2243	413	25			
R57/6 ..	2874	4138	2321	603	63	15284	0.63+0.30+0.07	11.5
I ..	2820	4199	2345	582	54			
III ..	2862	4119	2338	616	64			
W54/5 ..	2659	4332	2504	475	30	3603	0.741+0.259	18.3
I ..	2809	4198	2353	586	55			
III ..	2601	4386	2461	516	36			
TG57/1 ..	2743	3983	2659	580	35	9254	0.537+0.413+0.050	5.5
I ..	2695	4182	2433	629	61			
III ..	2695	4182	2433	629	61			
W54/2 ..	2459	4354	2665	501	21	3753	0.7287+0.2713	6.8
I ..	2658	4175	2460	644	63			
III ..	2401	4410	2613	540	36			
W54/1 ..	2559	4276	2501	625	39	3314	0.535+0.385+0.080	0.8
I ..	2643	4173	2470	650	64			
III ..	2500	4300	2549	602	49			
TG57/5 ..	2970	3472	2635	833	90	8923	0.51+0.43+0.06	2.5
I ..	2541	4152	2544	693	71			
III ..	3025	3740	2366	748	121			
O55/1 ..	2005	3980	3027	906	81	1721	0.7205+0.2795	42.9
I ..	2052	3987	2906	941	114			
III ..	1980	4049	2960	910	100			
A53/1 ..	2079	3513	3495	857	56	11822	0.519+0.403+0.078	42.9
I ..	1991	3959	2951	978	121			
III ..	1998	3996	2946	948	112			
W54/9 ..	1619	3972	3277	1054	78	3463	0.718+0.282	37.2
I ..	1785	3845	3105	1141	150			
III ..	1600	4016	3304	984	96			
O57/6 ..	1078	4260	3988	635	39	9001	0.445+0.455+0.100	1.1
I ..	1709	3796	3160	1170	162			
III ..	1156	4114	4029	665	30			
O57/1 ..	1199	3712	3649	1286	154	9424	0.447+0.447+0.106	226.4
I ..	1419	3572	3372	1414	223			
III ..	1225	3710	3649	1272	144			

Localities : A, Audley. R, LP, La Perouse and Malabar. O, Oatley. E, Evans Head. TG, Tea Gardens. W, Warrah. Gr, Grampians.

TABLE 1.—Continued.
Astroloma pinifolium.—Continued.
 Tetrad Segregation Data.—Continued.

Plant and Hypothesis.	Tetrad Type Proportions. % × 100.					N.	(p + q) or (x + y + z).	χ ² .
	r ₀ .	r ₁ .	r ₂ .	r ₃ .	r ₄ .			
E51/2 ..	454	3128	4876	1424	118	1608	0·5594 + 0·4406	136·4
I ..	957	<i>3086</i>	<i>3644</i>	1913	377			
III ..	484	2948	4968	1474	121			
O57/5 ..	940	2464	3698	2351	547	6466	0·22 + 0·67 + 0·11	2·55
I ..	742	2719	3736	2280	522			
III ..	841	2668	3566	2300	625			
O55/8 ..	3169	4260	2191	351	29	2392	0·29 + 0·46 + 0·25	26·5
I ..	3249	<i>4218</i>	<i>2053</i>	444	36			
III ..	3136	4368	2081	390	25			
R57/11 ..	3630	3918	1974	434	43	9098	0·56 + 0·39 + 0·05	3·1
I ..	<i>3452</i>	4206	2107	<i>390</i>	36			
III ..	3600	3960	1929	462	49			
GR58/1007 ..	3028	5946	1026	0	0	2758	q = 0·200	
Gr58/1005 ..	1229	7149	1614	4	4	2571	q = 0·260	
Gr58/1008 ..	660	1657	7579	33	71	2408	q = 0·430	
Gr58/1001 ..	431	2082	7416	5	66	1974	q = 0·430	

Localities: A, Audley. R, LP, La Perouse and Malabar. O, Oatley, E, Evans Head. TG, Tea Gardens. W, Warrah. Gr, Grampians.

For hypothesis I (independence) expected frequencies are given in *italics* where they are below observed frequencies.

In the collection of the data, the greatest possible care has been taken to avoid biologically meaningless sources of error. In slide preparation, anthers were dissected in a drop of stain-mountant on the slide under binocular magnification, to remove at least 90% of the pollen. The drop of mountant used was limited so that no exudation occurred at the edges of the cover slips, which might lead to a loss of the small nullads. Finally, within-slide sampling error has been avoided in most cases by scanning the whole area of each slide. In the data given for the Grampians plants of *Ast. pinifolium* a sectorial sampling method was used.

THE ANALYSIS.

Three different hypotheses have been examined. These are: (I) The survival or death of any microspore is independent of the fates of its three sister microspores in the same tetrad. (II) There is competition for survival between the four microspores in each tetrad. (III) The fates of the four microspores in each tetrad are related to the meiotic divisions.

I. *The hypothesis of independence.*

With independence, the observed frequencies of the five classes of tetrad should conform to the terms of the binomial (p + q)⁴, where q is the prospect that any microspore will survive, and p (=1 - q) is its prospect of abortion.

Writing a₀...a₄ for the observed frequencies of the five tetrad types, their summation being N, the total sample, and r₀...r₄ for the corresponding proportions, summing to unity, the observed value of q is

$$q = \frac{4a_4 + 3a_3 + 2a_2 + a_1}{4N}$$

Using values of q obtained directly from the data, expected frequencies e₀...e₄, based on the hypothesis of independence, have been calculated. Comparison of the observations with the expected frequencies (Hypothesis I) are presented in Tables 1, 2 and 3. Only one-quarter of the plants of eastern *Ast. pinifolium* show an acceptable fit to the hypothesis. The distributions of tetrad-type frequencies in the Grampians

plants are remote from the binomial form, and in *Acrotriche fasciculiflora*, all thirteen plants examined show an unsatisfactory fit.

The hypothesis of independence does not provide a generally satisfactory explanation of the segregation behaviour in either species. In the eastern *A. pinifolium*, however, it seems probable that the causes of tetrad segregation are occasionally capable of simulating independence.

TABLE 2.
Acrotriche fasciculiflora.
Tetrad Segregation Data.

Plant and Hypothesis.	Tetrad Type Proportions. % × 100.					N.	Parameters (p+a) or (x+y+z).	χ^2 .
	r ₀ .	r ₁ .	r ₂ .	r ₃ .	r ₄ .			
54/11	9603	332	62	3	0	3378		
I	9544	448	8	0	0		0.9884 + 0.0116	213.8
III	9604	333	62	1	0		0.980 + 0.017 + 0.003	0.03
54/12	9012	739	244	5	0	4008		
I	8813	1132	54	1	0		0.9689 + 0.0311	318.9
III	9025	722	242	9	0		0.950 + 0.038 + 0.012	0.05
54/8	1824	4368	3231	561	16	1891		
I	2214	4055	2784	850	97		0.686 + 0.314	59.8
III	1806	4420	3172	572	30		0.425 + 0.520 + 0.055	1.68
54/9	1331	4429	3775	459	6	1778		
I	1967	3947	2969	993	124		0.666 + 0.334	158.1
III	1369	4403	3799	417	12		0.370 + 0.595 + 0.035	0.76
54/7	1010	4657	3872	461	0	2278		
I	1841	3878	3064	1076	142		0.655 + 0.345	276.4
III	1156	4250	4144	438	12		0.34 + 0.625 + 0.035	17.2
54/1	1349	3678	4074	888	11	2602		
I	1646	3753	3208	1212	174		0.637 + 0.363	120.0
III	1122	4087	4090	671	30		0.335 + 0.610 + 0.055	43.8
54/3	974	4273	3980	773	0	1294		
I	1636	3746	3216	1227	176		0.636 + 0.364	104.2
III	1069	4009	4150	736	36		0.327 + 0.613 + 0.060	4.2
54/4	833	4395	4155	616	0	1752		
I	1638	3746	3216	1227	176		0.636 + 0.364	214.9
III	973	4012	4415	579	20		0.312 + 0.643 + 0.045	12.7
54/2	822	4183	4300	655	40	2979		
I	1546	3678	3282	1302	196		0.627 + 0.373	348.1
III	961	3968	4406	640	25		0.31 + 0.64 + 0.05	13.1
54/5	784	4067	4579	606	0	2326		
I	1516	3654	3303	1327	200		0.624 + 0.376	345.3
III	824	3846	4736	577	18		0.287 + 0.670 + 0.043	5.9
54/10	677	3862	4602	854	5	2084		
I	1375	3533	3402	1456	234		0.609 + 0.391	267.2
III	784	3752	4769	670	25		0.28 + 0.67 + 0.05	12.6
54/13	600	4122	4277	966	35	1999		
I	1349	3498	3403	1471	239		0.607 + 0.393	218.7
III	812	3733	4732	786	36		0.285 + 0.655 + 0.060	32.7
54/6	409	3232	5274	1067	18	1640		
I	1086	3223	3587	1775	329		0.574 + 0.426	278.1
III	484	3168	5448	864	36		0.22 + 0.72 + 0.06	10.8

Contingencies :

Total, $\chi^2 = 798.1$ (excluding 54/11 and 54/12).

Between plants, $\chi^2 = 542.5$.

Within plants, $\chi^2 = 255.6$.

Within plant data not presented in table.

All sets of data are from counts of two flowers.

(The full tetrad classes, being generally very small, have been grouped with the triad classes for the calculation of the contingency χ^2 , leaving a total of 22 degrees of freedom.)

II. The hypothesis of competition.

There are many reasons for the inference that cytoplasmic gradients may constitute the primary system of control in pollen development in the Styphelieae (Smith-White, l.c.). It is possible that the frequent occurrence of tetrad segregation in the tribe is

dependent upon cytoplasmic differentiation. Such a system would necessarily involve competition between sister microspores for cytoplasmic necessities. The occurrence of a competition effect, however, would not exclude a nuclear or even a chromosomal mechanism.

Competition between sister microspores would cause deviations from the theoretical frequencies calculated on the basis of independence. Such competition may be either positive or negative.

Negative competition or assistance would occur if, when some microspores survive, others in the same tetrad have an increased prospect of survival. A similar situation would exist if the death of a microspore increased the prospect of death of other

TABLE 3.
Maximum Likelihood Approximations to Independence and to the Trinomial Square Form.
East Coast Populations of *Astroloma pinifolium*.

Hypothesis.	Population.	Number of Plants showing χ^2 Values Equivalent to Probabilities of							Total Plants.
		<0.01	0.01-0.05	0.05-0.10	0.10-0.50	0.50-0.90	0.90-0.95	0.95-1.0	
I. Independence ($p+q$) ⁴	Oatley ..	13	—	—	3	1	2	2	21
	Warrah ..	7	—	1	—	—	—	—	8
	Evans Head ..	6	—	—	—	—	—	—	6
	Malabar ..	11	3	—	2	2	—	—	18
	Tea Gardens ..	2	—	—	1	—	—	1	4
	Totals ..	39	3	1	6	3	2	3	57
III. Trinomial square ($x+y+z$) ³	Oatley ..	3	3	—	4	6	2	3	21
	Warrah ..	—	—	—	2	5	1	—	8
	Evans Head ..	—	—	—	3	2	1	—	6
	Malabar ..	1	3	—	3	6	3	2	18
	Tea Gardens ..	2	—	—	1	—	—	1	4
	Totals ..	6	6	0	13	19	7	6	57

members of the tetrad. Just this effect has been observed in an unusual strain of *Petunia* studied by Levan (1942). It leads to an excess of nullads and full tetrads, and a deficiency of the other classes, compared with independence. Levan infers that dying pollen grains produce toxic degenerative substances which have adverse effects on the other members of the tetrad.

Conversely, positive competition implies that where some microspores develop, others in the same tetrad are at a disadvantage—necessary substances may be in limited supply, and may have non-uniform distribution within the mother cell.

Comparison of the data in Table 1 with binomial expectancy shows that in eastern *Ast. pinifolium*, in those plants where the data do not fit the hypothesis of independence, there is no consistent surplus or deficiency in the dyad and monad classes as compared with the binomial expectancy. There is no consistent indication of either positive or negative competition within tetrads.

In the data for *Acr. fasciculiflora* given in Table 2, however, all thirteen plants showed a marked excess of dyads over binomial expectancy, nine plants show an excess of monads, and all other classes in all plants, with the minor exception of nullads in plant 54/12, are deficient. The consistency of the thirteen sets of data suggests very strongly that competition between microspores does operate, and further analysis is justified.

If q_1 and p_1 , respectively, represent the prospects that a first microspore will survive or abort, and if q_2 , q_3 , q_4 and p_2 , p_3 , p_4 are the prospects for survival or abortion of a second microspore following the survival of a first, of a third following the survival of two, and of the fourth following the survival of the other three, the theoretical

frequencies of the five tetrad categories will be:

$$e_0 = p_1^4$$

$$e_1 = q_1 (p_1^3 + p_1^2 p_2 + p_1 p_2^2 + p_2^3)$$

$$e_2 = q_1 q_2 (p_1^2 + p_2^2 + p_3^2 + p_1 p_2 + p_1 p_3 + p_2 p_3)$$

$$e_3 = q_1 q_2 q_3 (p_1 + p_2 + p_3 + p_4)$$

$$e_4 = q_1 q_2 q_3 q_4$$

The values q_1 , q_2 , q_3 and q_4 can be related such that

$$q_2 = f_1 q_1, \quad q_3 = f_2 q_2, \quad q_4 = f_3 q_3$$

$$\text{and } f_2 = a f_1, \quad f_3 = c a f_2 = c a^2 f_1.$$

Dropping the subscript to f_1 ,

$$q_2 = f q_1, \quad q_3 = a f^2 q_1, \quad q_4 = c a^3 f^3 q_1.$$

In this formulation, there are four parameters, q_1 , f , a and c , and the last three measure or determine the increasing severity of competition between first and second, second and third, and third and fourth microspores. Since there are at most four degrees of freedom in any set of data, statistical testing of the formulation is not possible. However, values for the parameters have been calculated for eleven sets of data from Table 2 (excluding plants 54/11 and 54/12). The following five sets are representative:

Plant	q	f	a	c
54/8367	.841	.958	.133
54/7436	.648	.650	.0077
54/1394	.951	.562	.0133
54/3441	.718	.794	.0000
54/6550	.732	.582	.0588

It will be noted that a is of the same order of size as f ; it may be slightly larger, or substantially smaller. The parameter c is very small, but lower values of c are not necessarily associated with low values of a or f . Competition acting against the third microspore is scarcely greater than that acting against the second, but that operating against a fourth microspore is very severe.

The very sharp increase in competitive effect operating against the survival of all four microspores, which is indicated by this analysis, suggests that competition does not conform to a simple pattern. It is consistent, however, with the operation of a cytoplasmic gradient (Text-figure 1A, B). If there is a gradient distribution of necessary cytoplasmic substances (stippling) and also variation in total amount, and if the plane of the first meiotic division tends to lie along this gradient, varying from position (a) to position (b) with a mean at (m), competitive effects consistent with those observed could result. It is perhaps significant that in *Astroloma* and in many other species of the Styphelieae the spindle of the first meiotic division is eccentric and laterally displaced (Text-figure 1C) and may be across a cytoplasmic gradient.

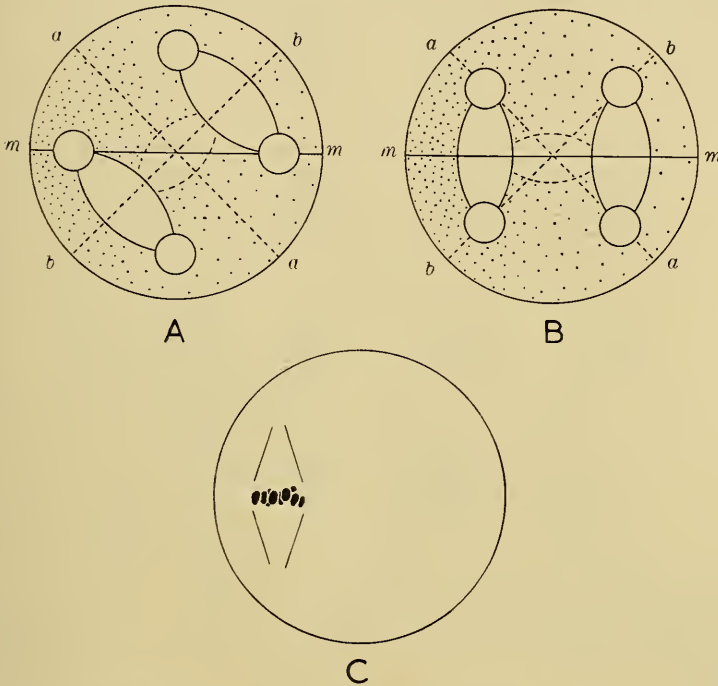
III. *The hypothesis of meiotic dependence.*

This hypothesis assumes that the tetrad segregations are related to and dependent upon the conditions of meiosis. Martin and Peacock (1959, pp. 272-278) have examined tetrad segregation in several species of *Leschenaultia* (Goodeniaceae). They find that, consequent upon low chiasma frequency and terminal chiasma localization, there is an appreciable frequency of univalents at first metaphase in pollen mother cells; loss and misdivision of univalents is responsible for some, but probably not all, of the observed pollen grain death.

In the Styphelieae it is not possible to recognize the plane of the first division in the mature pollen tetrads. This plane, however, may still be considered to divide the pollen mother cell (PMC) into two half-mother cells ($\frac{1}{2}$ MCs). Each $\frac{1}{2}$ MC would then be capable of yielding one of three possible results—neither, one only, or both its daughter microspores might survive. The prospects of these three possible results will be designated by the symbols x , y and z respectively.

With independence, there would arise the identities $x = p^2$; $y = 2pq$; $z = q^2$; and $(x + y + z)^2 = (p + q)^4$.

Independence, however, might operate between, but not within, half-mother cells and the tetrad-type frequencies would then fit the expansion of the trinomial $(x + y + z)^2$. This relationship, which will be referred to as the "trinomial square", is illustrated in Text-figure 2. Recognition of the plane of the first division, if possible, would permit the separation of the terms y^2 and $2xy$ in the theoretical frequency of dyads. This hypothesis of independence between $\frac{1}{2}$ MCs is hypothesis III in Tables 1, 2 and 3.



Text-figure 1.—The effect of competition in a cytoplasmic gradient. The gradient is represented by stippling. Variation in total supply of the gradient substances is not indicated. The orientation of the division spindles varies, with a mode as in B. Orientation as in A would yield monads or triads, according to the quantitative supply of gradient substance. Orientation as in B would yield dyads rather than monads. Cf. text. In C, the spindle eccentricity characteristic of the family is illustrated.

In order to test the data in Tables 1, 2 and 3 against the hypothesis, maximum likelihood estimates of x , y and z have been approximated, using a method of iteration described by Kempthorne (1957, pages 172-177), the iteration being repeated only until a satisfactory χ^2 value was obtained, or until it became obvious that a satisfactory χ^2 value was unobtainable. In many of the sets of data, the initial estimates, obtained from the equations

$$y = \sqrt{2r_0 + r_1 + r_2 - 2\sqrt{r_0}}, \quad x = \frac{r_1}{2y_1}, \quad z = 1 - x - y$$

have given acceptable χ^2 values, and no further iteration has been carried out.





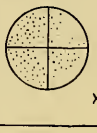

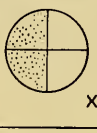
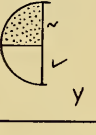
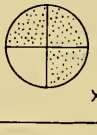
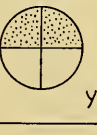

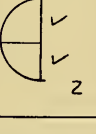



The observed frequency distributions obtained for eastern *Ast. pinifolium* are surprisingly consistent with this third hypothesis (Tables 1, 3). By contrast, the tetrad-type distributions obtained for the Grampians population of the species are remote from the form, and it must be inferred that the direct causes of segregation are quite different.

In *Acr. fasciculiflora*, four plants among thirteen give an excellent fit to the hypothesis, two show a barely acceptable fit, and seven show a very bad fit.

Within-Plant Variation.

Significant variation occurs between flowers within plants in probably all plants of both species. Intraplant variation, however, generally seems to be of lesser degree than interplant variation, and this feature is clearly shown by Text-figure 3. In Table 2,

THE TRINOMIAL SQUARE FORM

Text-figure 2.—The trinomial square form.

x , y and z are the prospects for the three possible results from a $\frac{1}{2}$ MC. With independence, the whole tetrad result becomes $(x + y + z)^2$.

the contingency χ^2 value for within-plant variation is only one-half that for between-plant variation, for equal degrees of freedom.

In Tables 4 and 5, data from individual flowers and anthers are given for two plants of *Ast. pinifolium* and are sufficient to illustrate the characteristics of intraplant variation. These two plants have been selected as representative of plants showing good and bad fit, respectively, to the trinomial square form.

In both plants there is significant variation, even between the five anthers of a single flower. In R57/6 (Table 4), the grand totals, flower totals, and individual anther sets, with one exception, all conform to trinomial squares. In A53/1, the data for flower 1 show an acceptable fit, but the other flowers do not, and the fit of the grand-total distributions is very bad.

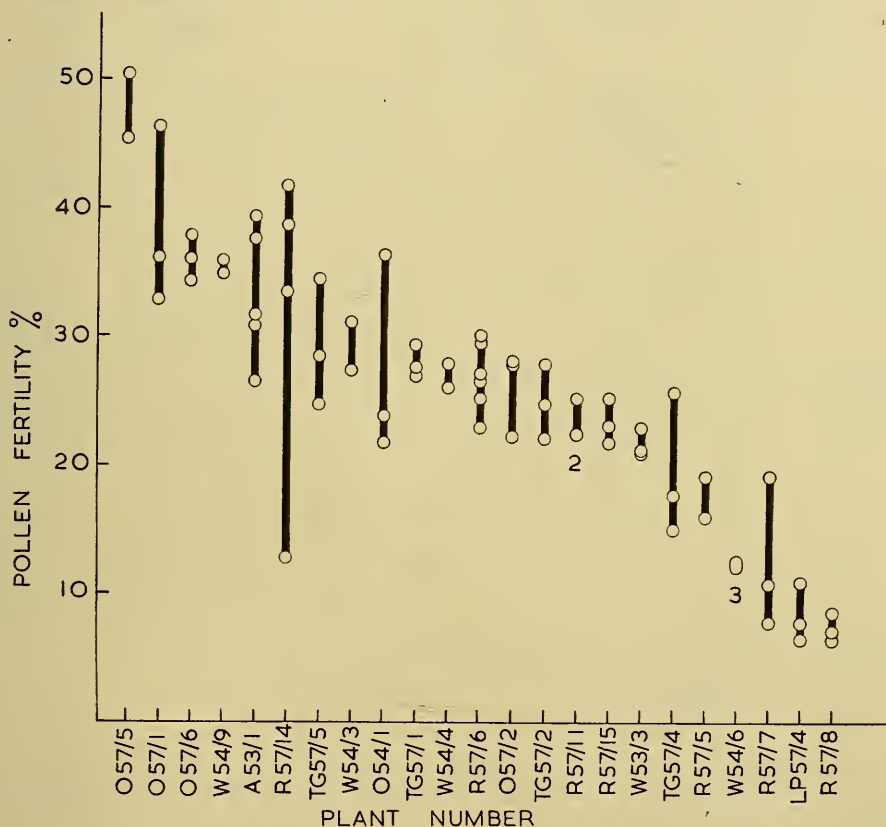
The data in Table 4 show two features which deserve comment. The first is that χ^2 values tend to be either very low, corresponding to probabilities above 0.5, or very high with probabilities below 0.01. The distribution of χ^2 values does not appear to be normal. The second feature is that, although the individual anther distributions all fit trinomial squares, they are significantly different, and yet the grand total distribution conforms to a "mean" trinomial square. It can be shown algebraically that the means of the individual terms of two different trinomial squares cannot be themselves the terms of a trinomial square. This contradiction in the data has not been resolved.

Intraplant variation in tetrad segregation is generally at a significant level, and must mean that the behaviour is subject to environmental modification. Interplant variation, however, is generally greater in degree, and this fact suggests that there are both genetical and environmental components involved. The existence of the

environmental effect, however, makes the interpretation of interplant variation difficult and hazardous.

The Meaning of the Trinomial Square Form.

The consistency of the fit to the trinomial square hypothesis in plants of eastern *Ast. pinifolium* must have significance in relation to the causes of tetrad segregation. This form has several implications which relate it to the conditions of meiosis (Text-figure 2).



Text-figure 3.—Variation in pollen fertility within and between plants. *Astroloma pinifolium*.

The values plotted are the q values for total microspore survival, each determination being from the five anthers of a flower. It is suggested that both genetic and environmental components affect pollen fertility.

The values x , y and z represent the prospects that neither, one only, or both the microspores derived from a second division $\frac{1}{2}$ MC shall survive. It is clear that to give the trinomial square form:

(i) The events leading to microspore failure or survival are operative or effective during the second division of meiosis.

(ii) The two $\frac{1}{2}$ MCs of each PMC must have similar prospects.

(iii) These prospects in the two $\frac{1}{2}$ MCs must be independent.

(iv) From (ii) and (iii) it follows that the first meiotic division must be strictly equational, both in chromosomal segregations or abnormal chromosome behaviour, and

in cytoplasmic differentiation. In particular, the cytoplasmic polarity present during the first division in *Leucopogon juniperinus*, which is demonstrated by the non-random segregation of univalents (Smith-white, 1948), and which is generally involved in the monad-type of pollen development in the Styphelieae (Smith-White, 1955, 1959), must be absent, or must be delayed, at least, until after the beginning of the second divisions.

TABLE 4.
Variation in Tetrad Segregation Within and Between Flowers.
Plant R57/6.

Flower.	Anther.	Tetrad Type Proportions. % × 100.					N.	q.	(x+y+z).	χ ² .	P.
		r ₀ .	r ₁ .	r ₂ .	r ₃ .	r ₄ .					
1	1	457	412	118	13	0	608	0.172	0.676+0.304+0.020	0.15	0.98
	2	290	458	219	31	2	576	0.247	0.538+0.426+0.036	0.10	0.99
	3	279	435	243	41	2	581	0.263	0.528+0.412+0.060	0.60	0.8
	4	287	399	241	66	7	544	0.277	0.54+0.37+0.09	0.19	0.98
	5	248	403	246	87	16	549	0.306	0.50+0.40+0.10	2.21	0.7
Totals	..	315	421	212	47	5	2858	0.252	0.562+0.375+0.063	1.16	0.85
2	1	339	356	246	59	0	675	0.256	0.52+0.40+0.08	19.1	0.001
	2	282	439	229	43	7	674	0.264	0.52+0.40+0.08	6.7	0.15
	3	272	431	221	69	5	605	0.276	0.52+0.41+0.07	2.4	0.5
	4	259	436	242	48	15	665	0.281	0.50+0.40+0.10	10.9	0.03
	5	256	423	246	72	3	601	0.286	0.51+0.42+0.07	1.85	0.75
Totals	..	283	416	237	58	6	3220	0.272	0.532+0.391+0.077	0.44	0.97
3	1	328	407	223	38	4	445	0.246	0.573+0.355+0.072	1.96	0.7
	2	295	423	231	49	2	529	0.260	0.55+0.39+0.06	1.11	0.8
	3	231	419	257	85	8	611	0.305	0.481+0.436+0.083	2.09	0.6
	4	222	413	274	82	9	559	0.311	0.47+0.44+0.09	0.11	0.99
	5	123	434	317	113	13	602	0.365	0.38+0.50+0.02	7.65	0.07
Totals	..	233	420	262	76	8	2746	0.301	0.483+0.435+0.082	1.67	0.7
Grand totals		278	419	237	60	6	3824	0.275	0.530+0.395+0.075	1.36	0.85

(Full tetrad classes have been grouped with triad classes.)

Contingencies: Total, $\chi^2=346.3$. 42 d.f.

Between flowers $\chi^2=71.3$. Heterogenous.

Contribution to total χ^2 by anthers 1-1 and 3-5=251.2.

The trinomial square form of frequency distribution does not necessarily imply but it does suggest that chromosomal or genetical segregations are involved. It is possible to devise model systems which are capable of giving this form. The conditions necessary are:

(i) There must be a substantial degree of chiasma localization, such that definite chromosome segments are always distal to the first chiasma, and always proximal to a second chiasma if such is formed. These segments will always show second division segregation and, like the differential segments of an *Oenothera* system, will be excluded from crossing over, and so protected from breakdown (Text-figure 4).

(ii) These segments, on different chromosomes, must carry all genes, or chromosome blocks, concerned in the tetrad segregation.

(iii) There must be at least two gene or chromosome block systems, A/a, B/b, . . . and A'/a', B'/b', . . . which may be either alternate or duplicate to each other in action. Each system must include two or more complementary genes or blocks, and all must be independent of one another in segregation.

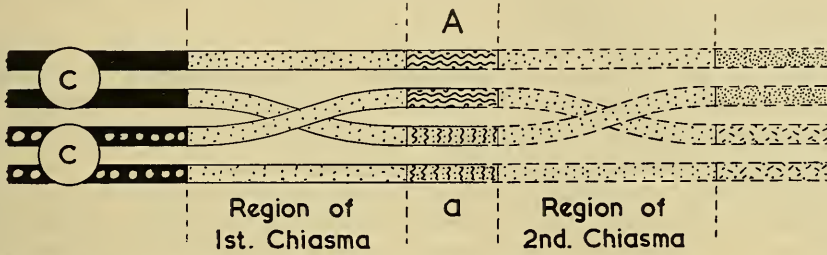
The minimum system possible is A/a B/b A'/a' B'/b' which would give

A B and A' B' systems duplicate, ($\cdot 125 + \cdot 625 + \cdot 250$)².

A B and A' B' alternate, ($\cdot 250 + \cdot 625 + \cdot 125$)².

A system A/a B/b C/c A'/a' B'/b' C'/c' would permit a wider range of trinomial squares.

Such an hypothesis of two gene or chromosome block systems is permitted by the apparent secondary polyploid constitutions of *Astroloma pinifolium* ($n=7, x=4$) and of *Acrotriche fasciculiflora* ($n=9$. Smith-White, 1955). However, the hypothesis would



Text-figure 4.—Diagram of a bivalent with chiasma localization.

Segments outside the chiasma regions are isolated from crossing over. The region distal to the first chiasma region would show regular reduction at second anaphase.

not seem to permit the degree of intraplant and environmental variation which has been described, unless it were associated with a very considerable effect of environment on chiasma frequency and chiasma localization.

Chromosome fragmentation is known to occur as a characteristic feature of meiosis in pollen mother cells of eastern *Ast. pinifolium*, and will be described in another paper. This abnormal meiotic behaviour may be associated with a system of chromosome

TABLE 5.
Variation in Tetrad Segregation Within and Between Flowers.
Astroloma pinifolium. Plant A53/1.

Flower.	Anther.	Tetrad-type Proportions.					N.	q.	Parameters (xyx).	χ^2 .	P.
		r_0 .	r_1 .	r_2 .	r_3 .	r_4 .					
1	1	332	395	239	33	0	461	0.242	0.54+0.41+0.05	6.1	0.10
	2	247	468	254	31	0	460	0.267	0.50+0.46+0.04	0.6	0.85
	3	254	448	270	28	0	496	0.268	0.51+0.45+0.04	2.9	0.40
	4	266	442	266	26	0	421	0.269	0.51+0.45+0.04	2.8	0.40
	5	231	453	265	49	2	433	0.282	0.50+0.45+0.05	1.0	0.80
Totals ..		267	441	259	33	0	2271	0.265	0.51+0.45+0.04	5.7	0.15
2	1	202	326	385	82	6	491	0.341	0.405+0.515+0.080	18.5	0.001
	2	185	322	368	118	6	475	0.360	0.39+0.52+0.09	14.5	0.004
	3	178	301	363	148	11	535	0.379	0.39+0.52+0.09	17.7	0.001
	4	134	308	429	124	5	396	0.390	0.32+0.58+0.10	9.0	0.01
	5	120	277	450	143	10	476	0.412	0.31+0.57+0.12	14.2	0.01
Totals ..		165	307	397	123	8	2373	0.376	0.343+0.550+0.107	82.9	0.000
3	1	140	315	381	149	15	549	0.363	0.35+0.52+0.13	7.6	0.10
	2	151	336	401	101	11	456	0.371	0.36+0.53+0.11	7.34	0.10
	3	135	336	405	172	10	398	0.386	0.36+0.53+0.11	4.78	0.30
	4	119	330	385	152	15	455	0.404	0.34+0.55+0.11	6.4	0.15
	5	102	303	458	131	7	413	0.409	0.32+0.58+0.10	9.1	0.01
Totals ..		129	323	403	133	12	2271	0.393	0.34+0.55+0.11	26.4	0.001
Grand totals		187	356	354	97	7	6915	0.345	0.38+0.53+0.09	130.1	0.000

Contingency : Total χ^2 .. 533.4. Heterogenous.
 Between flowers 439.3. Heterogenous.
 Within flowers 94.1. Heterogenous.

segregation similar to the model just described. Fragmentation does not occur in Grampians *Ast. pinifolium* or in *Acrotriche fasciculiflora*, and it must therefore constitute an additional complication rather than a primary cause of pollen tetrad segregation in the Styphelieae.

SUMMARY AND CONCLUSIONS.

An analysis of pollen tetrad segregation in *Astroloma pinifolium* and *Acrotriche fasciculiflora* has been attempted.

In eastern *Ast. pinifolium*, segregations generally fit a trinomial square form, and occasionally fit the hypothesis of independence. Tetrad segregations in a population of the same species from south-west Victoria (Grampians) are very different.

In *Acrotriche fasciculiflora*, segregations occasionally fit the trinomial square form, but more often show significant departures from this form.

The trinomial square form of tetrad frequency distribution requires strict equationality of the first meiotic division, and independence between the two second divisions in each mother cell, and a genetic or even chromosomal mechanism may be involved.

Deviations from the trinomial square form could be determined by loss of strict equationality of the first division. In the case of genetic or chromosomal mechanisms this would require a shift in regions of chiasma localization.

Consistent departures from expectations based on independence in *Acrotriche fasciculiflora* suggest that cytoplasmic conditions may be in part responsible for pollen death within tetrads. In particular, a gradient distribution of cytoplasmic substances may be involved.

Highly significant and very substantial intraplant variation in tetrad type frequencies occur, showing the behaviour to be sensitive to environmental modification. Until environmental effects can be evaluated, attempts to explain interplant and interpopulation differences are hazardous. Acceptable model systems must permit substantial environmental modification.

It is possible that both chromosomal and cytoplasmic systems operate as partial causes of segregation behaviour in the Styphelieae.

Literature Cited.

- KEMPTHORNE, O., 1957.—*An Introduction to Genetic Statistics*. John Wiley & Sons, Inc., N. York.
- LEVAN, A., 1942.—A gene for the remaining in tetrads of ripe pollen in *Petunia*. *Hereditas*, 28: 429-435.
- MARTIN, P. G., and PEACOCK, W. J., 1959.—Pollen tetrad patterns in *Leschenaultia*. *Proc. Linn. Soc. N.S.W.*, 84: 271-277.
- SMITH-WHITE, S., 1948.—Polarised segregation in a stable triploid. *Heredity*, 2: 119-129.
- , 1955.—Chromosome Numbers and Pollen Types in the Epacridaceae. *Aust. J. Bot.*, 3: 48-67.
- , 1959.—Pollen Development Patterns in the Epacridaceae. Pres. Address. *Proc. Linn. Soc. N.S.W.*, 84: 8-35.